RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

September 6-7, 2001

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

CONTENTS

Note: The latest Human Gene Transfer Protocol List can be found at the Office of Biotechnology Activities' Web site at <http://www4.od.nih.gov/oba/RDNA.htm>.

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
MINUTES OF MEETING[1]
September 6-7, 2001

The 83rd meeting of the Recombinant DNA Advisory Committee (RAC) convened at 8:30 a.m. on September 6, 2001 at the National Institutes of Health (NIH), Building 45, Natcher Conference Center, Conference Room D, 9000 Rockville Pike, Bethesda, MD 20892. Dr. Claudia A. Mickelson (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public from 8:30 a.m. until 6:20 p.m. on September 6 and from 8:30 a.m. until 12:30 p.m. on September 7. The following individuals were present for all or part of the meeting:

Committee Members

C. Estuardo Aguilar-Cordova, Harvard Gene Therapy Initiative
Dale G. Ando, Cell Genesys
Xandra O. Breakefield, Massachusetts General Hospital
Theodore C. Friedmann, University of California, San Diego
Jon W. Gordon, Mount Sinai School of Medicine
Jay J. Greenblatt, National Cancer Institute (NCI), NIH
Nancy M.P. King, University of North Carolina, Chapel Hill
Sue L. Levi-Pearl, Tourette's Syndrome Association
M. Louise Markert, Duke University Medical Center
Claudia A. Mickelson, Massachusetts Institute of Technology

Executive Secretary

Amy P. Patterson, NIH

Ad Hoc Reviewers/Speakers
John M. Coffin, Tufts University School of Medicine, NCI
Martin Friedlander, Scripps Research Institute
James B. Kaper, University of Maryland School of Medicine
Michael J. Mann, University of California, San Francisco
Glen R. Nemerow, Scripps Research Institute (via conference call)
John Reed, Burnham Institute (via conference call)
Charles M. Rudin, University of Chicago Medical Center
Joan Schwartz, National Institute of Neurological Disorders and Stroke
James E. Talmadge, University of Nebraska Medical Center
Jiing-Kuan Yee, City of Hope National Medical Center and Beckman Research Institute
John A. Zaia, City of Hope National Medical Center and Beckman Research Institute
Marco Zarbin, University of Medicine and Dentistry of New Jersey (via conference call)

Nonvoting/Agency Representatives
Philip Noguchi, U.S. Food and Drug Administration (FDA)
Stephanie L. Simek, FDA

[1] The Recombinant DNA Advisory Committee is advisory to the National Institutes of Health (NIH), and its recommendations should not be considered as final or accepted. The Office of Biotechnology Activities should be consulted for NIH policy on specific issues.

NIH Staff Members
Peter Dudley, National Eye Institute
Kelly Fennington, Office of the Director (OD)
Laurie Harris, OD
Robert Jambou, OD
Robert Lanman, OD
Kathryn Lesh, OD
Marin Mautino, National Human Genome Research Institute
Barbara McDonald, OD
Cheryl McDonald, OD
Akira Miyaz, National Heart, Lung, and Blood Institute
Richard Morgan, National Cancer Institute
Mary Nuss, National Institute of Allergy and Infectious Diseases (NIAID)
Marina O'Reilly, OD
Alexander Rakowsky, OD
Stephen M. Rose, NIAID
Eugene Rosenthal, OD
Joan Schwartz, OD
Thomas Shih, OD
Allan Shipp, OD

Others

Approximately 95 individuals attended this 2-day RAC meeting. A list of attendees appears in Attachment II.

I. Call to Order and Opening Remarks/Dr. Mickelson

Dr. Mickelson, RAC Chair, called the meeting to order at 8:30 a.m. on September 6, 2001. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* was published in the *Federal Register* on August 14, 2001 (66 FR 42673). Issues to be discussed by the RAC at this meeting included reviews of four gene transfer protocols, the quarterly data management report, two clinical protocol updates, communication of issues raised by preliminary RAC review, three proposed amendments to the *NIH Guidelines*, an update on the December 2001 Institutional Biosafety Committee (IBC) policy conference, updates on the Scope of the *Guidelines* and Vaccine Working Groups, and a presentation about implications for the RAC regarding donation of ooplasm as a treatment for infertility.

Dr. Patterson read aloud the statement regarding the NIH Rules of Conduct and Conflict of Interest.

A list of abbreviations and acronyms appears in Appendix III.

II. Minutes of the June 14-15, 2001 Meeting/Dr. Gordon and Ms. Levi-Pearl

A. Committee Motion 1

As moved by Dr. Gordon and seconded by Dr. Greenblatt, the RAC unanimously accepted the June 14-15, 2001 minutes by a vote of 9 in favor, 0 opposed, and 0 abstentions.

III. Clinical Protocol Updates

Dr. Patterson noted that “Clinical Protocol Updates” is a new part of the RAC agenda that will be a regular feature.

A. A Phase II Study of Autologous CD4-Zeta Gene-Modified T Cells in HIV-Infected Patients with Undetectable Plasma Viremia on Combination Antiretroviral Drug Therapy (Protocols 9709-213 and 9805-253)/Dr. Ando

Dr. Ando presented data from protocols 9709-213 and 9805-253. In each protocol, T cells were obtained from HIV positive research participants, modified by inserting the gene for the CD4-zeta receptor (utilizing a retroviral vector), and reinfused. The research participants of Protocol 9709-213 had discontinued antiretroviral therapy and had undetectable plasma viremia. The goal of the study was to prolong undetectable plasma viremia beyond two months. After the development of highly active anti-retroviral therapy (HAART), the trial was terminated because discontinuing such effective therapy, even for short periods of time, could no longer be justified.

Protocol 9805-253 extended the work of 9709-213. Research participants on HAART were infused to determine if the addition of the transduced autologous T cells would result in greater clearance of HIV viral reservoirs compared with a control group. The study results showed no significant drop in viral reservoirs in the treatment group when compared to the control. No significant adverse events (AE) were noted. The study was discontinued due to insufficient efficacy to warrant moving into a larger Phase III study. The study was valuable because it provided a safety baseline that could be applied to other trials using similar T-cell therapies. Dr. Ando noted that one such proposed trial (protocol 0107-488) would be reviewed later in the meeting.

B. A Multicenter, Double-Blind, Placebo-Controlled Phase II Study of Aerosolized tgAAV-CF in Cystic Fibrosis Patients with Mild Lung Disease (Protocol #0006-404)/Ms. King and Dr. Markert

Ms. King and Dr. Markert reported on the status of Protocol 0006-404 which was reviewed at the September 2000 RAC meeting. This Phase II study of aerosolized tgAAV-CF in cystic fibrosis (CF) patients proposed the administration of three doses of $1x10^{13}$ particles by inhalation once a month. The primary objective was to determine safety as measured by the number and severity of AEs. Secondary measures included pulmonary function tests, high-resolution computerized tomography, sputum changes, changes in neutralizing antibodies to adenoassociated virus (AAV), and a variety of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) assessments in lung cells obtained by bronchoscopy. The study design required that adult participants be treated before participants younger than 18 years were enrolled.

When the protocol was reviewed, the RAC indicated an interest in evaluating and reviewing the data from adult research participants prior to the inclusion of research participants younger than 18 years old in the study. The RAC also suggested an increase in the number of research participants in the placebo arm. Since no severe AEs were seen in the adult participants, the data safety monitoring board (DSMB) has approved the enrollment of research participants ages 15 to 17. Increasing the number of placebo participants was also implemented by the sponsor. As required by the protocol, consent monitoring will be initiated for the 15- to 17-year-olds. Ms. King suggested that the RAC ask for an update on the progress of the consent monitoring process.

IV. Discussion of Human Gene Transfer Protocol #0107-485: Purging of Autologous Stem-Cell Sources With bcl-x_s Adenovirus for Women Undergoing High-Dose Chemotherapy for Stage IV Breast Carcinoma

Principal Investigator:	Michael F. Clarke, M.D., University of Michigan, Ann Arbor
Sponsor:	None
RAC Reviewers:	Drs. Friedmann and Gordon and Ms. King

Ad Hoc Reviewers: John Reed, M.D., Ph.D., Burnham Institute *(not present)*;
Charles M. Rudin, M.D., Ph.D., University of Chicago Medical Center; and
James E. Talmadge, Ph.D., University of Nebraska Medical Center

A. Protocol Summary

High-dose chemotherapy (HDCT) combined with autologous bone marrow transplantation (BMT) is a treatment for metastatic cancer, including breast cancer and neuroblastoma. However, the marrow of such patients is often contaminated with tumor cells. The principal investigator (PI) recently discovered that a recombinant adenovirus vector containing a bcl-x_s minigene (a dominant negative inhibitor of the bcl-2 family), called the bcl-x_s adenovirus, is lethal to cancer cells derived from epithelial tissues, but not to normal human hematopoietic cells.

When breast cancer cells and hematopoietic cells were transduced with the bcl-x_s adenoviral vector, the cancer cells were selectively killed by the vector. In studies in mice, hematopoietic cells that had been exposed to the vector were able to reconstitute the bone marrow of mice exposed to lethal doses of gamma irradiation. These studies suggest that adenovirus suicide vectors may provide a simple, safe, and effective method to selectively eliminate cancer cells derived from epithelial tissue that contaminate bone marrow to be used for autologous BMT. The researchers propose to initiate a Phase I clinical trial to test the safety of this concept in humans. The research participants are women with breast cancer undergoing high dose chemotherapy and autologous BMT.

B. Reviews by RAC Members and Ad Hoc Reviewers

Four RAC members voted for public review of this protocol. Drs. Friedman and Gordon and Ms. King submitted written reviews, as did *ad hoc* reviewers Drs. Reed, Rudin, and Talmadge. Dr. Clarke responded to these reviews in writing and during this meeting.

Dr. Friedmann requested RAC discussion of this protocol because of the following issues: (1) the rationale for the *in vivo* tumorigenesis studies with spiked samples, (2) the need to discuss the value of this study in the context of the current uncertainty regarding HDCT-BMT as an approach to the treatment of breast cancer, and (3) the pharmacodynamics and biodistribution of the vector, assays to demonstrate absence of replication competent virus, and the effects on other organs in the body, including the liver. During the meeting Dr. Friedmann also raised concerns about the possibility of endothelial apoptosis, the effect on the vascular system, and any thrombolytic or thrombotic activity.

Dr. Gordon asked the investigator to discuss the animal experiments assessing bone marrow reconstitution efficiencies with and without *ex vivo* exposure to the vector. He expressed concerns about potential toxicities, such as those related to surface interactions; *in vivo* toxicity related to unbound adenovirus; viral genes that remain in the vector; and the possibility of an immune response *in vivo*. He asked the investigator to discuss the potential toxicities of adenoviral vectors employed *in vivo* and *ex vivo*. Dr. Gordon also noted that, compared with some other protocols, these hypothetical mechanisms of toxicity may be of relatively less concern since this protocol employs an *ex vivo* exposure to the adenoviral vector rather than a direct infusion into the research participant.

Ms. King raised issues related to potential toxicities and the appropriateness of moving from preclinical to clinical studies at this point and using this design. She asked about the dose-escalation design and the anticipated risk:benefit balance for each dosing cohort; the data monitoring plan; and selection of the study population. She also offered suggestions about the informed consent document and process. She noted issues associated with the study population and the need for additional information on the alternative therapies available to these women. Because participants are in advanced stages of disease, the study population would not be eligible for other trials, setting up an expectation of benefit. To help prevent this

misconception, Ms. King suggested using a study population that would be eligible to take part in other trials. She also indicated an interest in reviewing the revised informed consent document.

Dr. Talmadge asked about the amount of vector to be used and the volume in which the stem cell product and the vector will be co-incubated. He was concerned that a high dosage of vector would be in a volume of freezing medium that could potentially cause CD34 toxicity. He also raised concerns about the surrogate testing to be used to identify tumor cell contamination of a stem cell product. He presented data comparing the clonigenic assay and RTPCR, which is much more sensitive, and suggested that a more sensitive assay might give more quantifiable data. Dr. Talmadge also questioned how the researchers intended to monitor for clinical or subclinical adenovirus infection and how it would be possible to differentiatate between the vector and a wild-type virus infection.

Dr. Rudin focused on the safety and potential efficacy of the adenoviral vector and the rationale for autologous transplantation in this study population. He noted that Dr. Clarke presented reasonable data to suggest that exposure of the peripheral stem cell harvest to this adenoviral vector is not likely to significantly delay engraftment and prolong the duration of neutropenia. He expressed concern about the presence of free vector particles in the stem cell preparation being reinfused into the research participant. Because of the proapoptotic function of the transgene, he was concerned about systemic toxicity. Regarding efficacy, Dr. Rudin stated that because the virus is very likely to infect essentially all breast cancer cells within the stem cell harvest, this approach is likely to be an effective purging methodology. With respect to autologous transplantation in this study population, he noted that there were no conclusive data to suggest that this was a superior treatment to standard dose chemotherapy. While the majority of cancer relapses may be due to failure of the chemotherapy to eradicate residual disease, relapse can also be caused by tumor contamination of bone marrow.

Dr. Reed, who was unable to attend the meeting, submitted a written review. His comments were presented and discussed by Dr. Rudin. He noted that bcl-x_2 adenoviral vector preparation should be free of replication-competent virus. If a research participant developed a concomitant wild-type adenovirus infection, it should be unlikely that the vector become competent. He expressed concern about the potential for transduction of endothelial cells to lead to vessel denudation, thrombosis, and possibly disseminated intravascular coagulation. Regarding the first point, Dr. Rudin agreed with Dr. Clarke that the frequency of wild-type adenovirus infections in this clinical population is actually low, making the production of significant vector titer by superinfection unlikely. As for the possibility of endothelial damage with this vector, Dr. Rudin noted that unlike hematopoietic progenitor cells, endothelial cells do not express high levels of the CAR receptor, thus, are difficult to infect with adenovirus. Therefore, he felt that vessel destruction and subsequent thrombotic events were unlikely to occur with this agent.

C. RAC Discussion

The following additional issues were raised by RAC members:

- The possibility of generating a replication-competent adenovirus that would contain the bcl-x_S gene and the potential for transmission to others.

- Given the limitations of detection of replication-competent adenovirus in vector preparations, Dr. Aguilar-Cordova asked Dr. Clarke about the potential for administering replication competent adenovirus to severely immunocompromised research participants.

- Whether enrollment should be limited to research participants with antibodies to adenovirus as a measure of safety.

- How the adenoviral vector was designed to replicate only in breast cancer cells.

- The consent document is lacking in a financial disclosure statement, a request for autopsy, and provision for research participants for whom English is not their first language.

D. Investigator Response

In response to Ms. King's concern about the appropriateness of this study population, Dr. Clarke responded that it will not be possible to predict the outcome in this population of patients if they cannot be enrolled. The investigators will inform prospective research participants of the available data on high-dose chemotherapy and any other treatment options for which they may be eligible. Dr. Clarke noted that the groups that would most likely benefit would be women with microscopic breast cancer who do not have bulk disease and children with neuroblastoma. However, because the proposed study is a Phase I safety trial, he did not believe that those two groups, which have other options available to them, should be the ones to establish the safety of this procedure.
In response to Dr. Friedmann's request for a further explanation of the design of vector, Dr. Clarke explained that the adenoviral E1 and E4 promoters had been replaced by estrogen, telomerase, and hypoxia responsive promoters.

With respect to concerns about any toxicity or proapoptotic activity with bcl-$x_{s,}$ Dr. Clarke noted that compared with other members of the bcl-2 family, bcl-x_s appears to be much less toxic to normal cells than to cancer cells. The mechanism for this difference is not well understood. With respect to concerns about superinfection, Dr. Clarke pointed out that wild-type adenovirus is cytotoxic by itself and the deletion of E1 inhibits the expression of the proapoptotic genes in the vector. It is not clear whether expression of the bcl-xs gene would add to the cytotoxicity of the virus.

Dr. Clarke acknowledged the need for updates and revisions to the informed consent document and assured the RAC that it would be done once they have input from all the different agencies.

E. Public Comment

No public comments were offered.

F. RAC Recommendations

Dr. Mickelson summarized the following RAC recommendations, suggestions, and comments as follows:

! Consideration should be given to modifying the inclusion criteria for the trial, either to substitute as potential participants women who would be otherwise eligible to receive high-dose chemotherapy with autologous transplantation in other studies at the PI's institution, or to add this population to the existing participant pool. In addition, consideration should also be given to modifying the inclusion criteria in order to retain as participants women who express interest in the study and who have given consent, but who then have no detectable residual tumor after standard induction chemotherapy.

! The baseline screening tests should include testing for adenoviral antibodies. Testing for adenoviral antibodies should be repeated at two months post-treatment.

! The freezing solution used for the vector product should be the standard University of Michigan freezing solution. Due to the presence of neutralizing or aggregating antibodies to adenovirus, consideration should be given to the substitution of clinical-grade human serum albumin for human serum.

! The protocol should include a plan for monitoring for viral vector shedding in the saliva, urine and stool of the treated participants. Consideration should be given to the development of a technique to distinguish wild-type from vector adenovirus.

- The development of any clinically significant adenoviral infection in treated participants should be a stopping rule for this trial.

- The investigators should consider the development and implementation of surrogate markers for detecting residual tumor cells in the stem cell samples.

- The informed consent document should contain a section disclosing any financial interests that any of the investigators may have in this protocol.

- Provisions should be made to ensure the adequacy of the informed consent process for study participants whose first language is not English and these provisions should be described in the informed consent document.

- The informed consent document should refer to the importance of autopsy in the event of a participant's death and that a request will be made to the next-of-kin.
- Many changes are needed in the informed consent document to clarify the purpose, design, risks, and potential benefits of the study. These revisions were not discussed in specific detail during the meeting. Following its submission, OBA staff will forward a copy of the final, IRB-approved informed consent document to the RAC.

G. Committee Motion 2

As moved by Ms. Levi-Pearl and seconded by Dr. Gordon these recommendations were approved by a vote of 10 in favor, 0 opposed, and 0 abstentions.

V. Discussion of Human Gene Transfer Protocol #0107-480: A Phase IIb, Randomized, Multicenter, Double-Blind Study of the Efficacy and Safety of Trinam™ (EG004) in Stenosis Prevention at the Graft-Vein Anastomosis Site in Dialysis Patients

Principal Investigator:	Valentin Fuster, M.D., Ph.D., Mount Sinai Medical Center
Sponsor:	Ark Therapeutics, Ltd., represented by Alan K. Boyd, B.Sc., M.B., Ch.B, FFPM; John Martin; and Seppo Ylä-Herttuala
RAC Reviewers:	Drs. Ando, Friedmann, and Juengst
Ad Hoc Reviewers:	Michael J. Mann, M.D., University of California, San Francisco Glen R. Nemerow, Ph.D., Scripps Research Institute (via conference call)

A. Protocol Summary

Patients undergoing hemodialysis for kidney disease often experience problems with the vascular access grafts required for dialysis. For hemodialysis access, a polytetrafluorethylene (PTFE) graft connecting the arterial and venous systems is surgically placed into the arm of the patient with kidney disease. The graft then can be used for venipuncture to allow hemodialysis to occur. Unfortunately, vascular access often fails because the graft-vein anastomosis (the site where the graft joins the vein) undergoes stenosis (narrowing) and the blood tends to clot. Approximately 40 percent of people with access grafts have problems with these grafts in the first 6 months after placement. Stenosis occurs when the wall of the vein becomes thicker than normal because of the proliferation of smooth-muscle cells (intimal hyperplasia) in the vein wall. Blood clots or stenosis of the vein can increase the risk of infection and the destruction of the graft. The hospital costs related to vascular access procedures in dialysis patients are estimated to be approximately $1.3 billion per year, and the total cost of dialysis complications to the U.S. health care system is estimated to be in excess of $2 billion per year.

Ark Therapeutics is developing a gene transfer product called Trinam™, which is proposed to prevent intimal hyperplasia at the graft-vein anastomosis in patients who require vascular access for hemodialysis due to kidney disease. Trinam™is an adenoviral vector expressing vascular endothelial growth factor D (VEGF-D) which is delivered locally to the adventitial surface of the graft-vain anastomosis by a collagen collar device. VEGF-D inhibits smooth-muscle cell migration and proliferation. VEGF-D acts on surface receptors on endothelial cells resulting in increased production of nitric oxide and prostacyclin which diffuse into the media of the blood vessel wall and counter the tendency for intimal hyperplasia to develop. In an *in vivo* rabbit model of intimal thickening in carotid arteries, adventitial delivery of VEGF-D using a silastic collar as a gene delivery reservoir prevented smooth muscle cell proliferation without evidence of new blood vessel formation.

The objective of the proposed study is to assess the effectiveness and safety of Trinam™ when applied to the graft-vein anastomosis in research participants with severe renal disease who require vascular access for dialysis. At the time the graft is placed in the arm, research participants will be randomized to either a single administration of Trinam™ or to no treatment. It is hypothesized that Trinam™ administration will result in less stenosis at the graft-vein anastomosis site compared with controls and, therefore, reduce the need for treatment of thrombosis and stenosis in dialysis patients. Approximately 210 research participants will be enrolled from 10 to 15 hospitals in the United States, and participants will be evaluated over a period of 6 months. The amount of virus to be used in this study has already been tested in pigs and was not found to cause any significant side effects.

B. Reviews by RAC Members and *Ad Hoc* Reviewers

Three RAC members voted to review this protocol publicly. Drs. Ando, Friedmann, and Juengst submitted written reviews, as did *ad hoc* reviewers Drs. Mann and Nemerow, to which the investigators responded in writing and during this meeting.

Dr. Friedmann asked for further explanation of the balance between the proliferative inhibitory function and angiogenic properties of VEGF-D. He questioned the appropriateness of a large Phase II study at the current stage of understanding of the technology and the large number of patients required for this study. He asked for any data on potential for transduction of kidney cells. He suggested that, in addition to the vascular loop study already conducted, the precise PTFE graft model also be tested in pigs.

Dr. Ando's review posed questions about the effects of neutralizing antibodies to the adenoviral vector or transgene expression given data indicating that adenoviruses can exacerbate intimal hyperplasia. He asked about the tests to be used to screen research participants for proliferative retinopathy. He suggested that more preliminary work may be needed before proceeding to a Phase II study, but was impressed with the DSMB being setup.

Because Dr. Juengst was not present at this RAC meeting, Dr. Mickelson presented his written review. While noting that the protocol was well designed and the informed consent document was thorough and written in lay language, Dr. Juengst questioned whether this process/product combination had been tested in Phase I human studies, and if not, why the trial is classified as Phase II.

Dr. Mann reviewed the state of the VEGF field emphasizing that the vascular protective and angiogenic functions of the factors are inseparable. The data supporting the vascular protective property come from a rabbit model using VEGF-A plasmid, and a silastic collar around a normal artery, thus differing in multiple ways from the protocol. In the rabbit preclinical studies, the silastic collar was associated with inflammation and increased neointimal hyperplasia. He expressed concern about the possibility of a similar effect aggravating the clinical outcome of the research participants. For this reason, he asked whether research had been done to determine whether adequate adenoviral vector (AdV) delivery could occur during intraoperative exposure and thereby obviate the need for longer term delivery via the collar. Dr. Mann recommended that the informed consent document more clearly state that studies have been performed only in a small number of animals. He expressed concern about the randomization scheme being a 2-to-1

ratio of treatment to control because of the small size of the control arm. Because of the difficulties in directly applying animal data to humans in this type of research, he stated that proceeding into a Phase II trial was justified as long as safety provisions (ongoing surveillance, DSMB, and long-term follow-up procedures) are incorporated into the study.

Dr. Nemerow (via conference call) noted that this protocol is interesting, addresses an important medical need, and represents a modest and achievable goal. The use of adenoviral vectors for local delivery may limit the vector toxicity associated with systemic delivery. However, the target, vascular endothelial cells, is relatively resistant to transduction, so the potential for the dissemination of excess vector warrants increased safety monitoring. He expressed concern about the lack of Phase I safety data. He asked whether the microscopic exam of pig tissue revealed any pathology, especially in the lymph nodes. Since VEGF-D appears to promote metastatic spread of tumor cells, he suggested a longer follow-up term than 6 months. He also suggested that vector dissemination be tested in plasma rather than peripheral blood mononuclear cells which are poorly transduced by adenoviral vectors. Also a small amount of the viral vector should be archived to provide a record for toxicity and infectivity measurements.

C. RAC Discussion

Additional concerns by RAC other members were as follows:

- The effect on the function of the stent if VEGF-D stimulates angiogenesis.
- The need to clarify the timeline between the completion and follow-up for the first two (unblinded) research participants and accrual into the second blinded portion of the trial.
- The risk of inflammation in the presence of preexisting immunity could easily be modeled in an animal study in which the animals are preimmunized to adenoviruses.
- The potential for biodistribution to major organs and clarification of the rationale for including research participants with grades 1 and 2 liver toxicities.
- The need to look for vector sequences in any tumors that occur.
- The fairness of accepting men who use contraceptives but excluding all women of reproductive age. Dr. Noguchi added that FDA would like to see more women represented in this trial and that exclusions should be based only on actual risk.

D. Investigator Response

Drs. Boyd and Martin responded to questions about the role of angiogenesis. Dr. Boyd stated that researchers found no evidence of angiogenesis outside of the target area in the pig study. Angiogenesis was seen under the collar at day 60, but it was present in both the treatment and control groups. Dr. Martin noted that angiogenesis of an adventitia is not necessarily a negative outcome. In a pig study, the development of small vessels in the adventitia was associated with a decrease in intimal hyperplasia.

Dr. Ylä-Herttuala responded to questions about the presence of neutralizing antibodies. In the pig study, all animals were naive, so no antibodies were present. In human trials, it is expected that some of research participants will have antibodies. One of the potential advantages of using the collar is that, during the first hours or days of VEGF-D administration, the virus will not come in contact with plasma components or neutralizing antibodies, making transduction at the target site relatively efficient.

Regarding screening for proliferative retinopathy, Dr. Boyd explained that their consultant on this issue recommended a fundoscopic examination documented by photography and fluorescein angiography.

Regarding the large size of this study, Dr. Boyd explained that it would be difficult to obtain any efficacy points from a smaller study. Dr. Mann's suggestion about using a 1:1, rather than a 2:1, randomization will be taken into consideration.

Regarding the timeline between the unblinded and blinded portions of the trial, Dr. Boyd explained that the purpose of the unblinded procedures was to accustom the surgeons to the procedure and to make sure that there are no subsequent complications. A safety review will be conducted at 1 month and if the research participants are well, and the surgeon is comfortable with the procedure, additional participants will be included in a blinded fashion. Researchers anticipate recruiting 10 centers to the study, all with gene transfer expertise and all with the appropriate vascular surgeons and pathologists.

Dr. Ylä-Herttuala stated that any inflammation seen to date has been attributable to the collagen collar rather than to the gene being infused using that device. Other viruses have been tested with the same collar, and the results show that the collagen attracts most of the inflammatory cells to the site.

Dr. Boyd agreed not to include research participants with grade 1 or 2 liver toxicities if the RAC so requests. He noted that renal and liver failure in the same individual is quite rare, so the size of the population being studied would not be affected significantly. He also agreed that women of child bearing potential should be included and asked for recommendations on modifying the protocol.

Regarding the follow-up period, Dr. Boyd noted that follow-up for this protocol will be an initial 6-month period, followed by another 6 months, with a total observation period of 1 year.

Dr. Boyd agreed that the researchers will analyze plasma in the biodistribution assay. He also agreed that the researchers will archive a small amount of the vector stock, in accordance with FDA procedures.

E. Public Comment

No public comments were offered.

F. RAC Recommendations

Dr. Mickelson summarized the following RAC recommendations, suggestions, and comments.

- Consideration should be given to conducting studies on pre-immunized animals to assess the effects of pre-existent immunity to adenovirus.
- Consideration should be given to changing the randomization scheme from 2:1 to 1:1.
- Consideration should be given to changing the exclusion criteria such that hepatic dysfunction at the level of NCI Common Toxicity Criteria as low as Grade 1 or 2 would be an exclusion criterion.
- The post-treatment follow-up period should be extended to 1 year to increase monitoring for any possible late-onset adverse events.
- Small amounts, or retention aliquots, of vector stocks should be archived for future reference. If infectivity and toxicities are observed in research participants, this would allow comparative assessment of the different vector lots.
- Screening tests should include evaluation for pre-existing antibodies to the vector.
- Because adenoviral vectors poorly infect peripheral blood leukocytes, vector dissemination may be missed if plasma is not examined. Therefore, plasma, as well as white blood cells, should be analyzed for the presence of vector sequences.
- Any tumors that develop in the research participants should be tested for the presence of vector sequences.
- The informed consent document should state that the first two research participants at each of the ten sites will be serving as the participants in the “proof of concept” portion of this clinical trial and that there will be a one-month interval for safety review before enrollment is expanded.
- The consent document should clarify that the surgeon placing the device will be “unblinded” with respect to that part of the protocol.

G. Committee Motion 3

As moved by Dr. Aguilar-Cordova and seconded by Dr. Greenblatt, these recommendations were approved the RAC by a vote of 9 in favor, 0 opposed, and 1 abstention.

H. Comment

Dr. Patterson noted that NIH requires that, within 20 days of enrolling the first patient, researchers submit a copy of the final protocol, institutional review board (IRB) and IBC approvals, the final informed consent document, and written responses to the letter from the RAC. If researchers have sound scientific or clinical reasons for not implementing RAC recommendations, those reasons should be explained in the response letter, which becomes part of the protocol record.

VI. Discussion of Human Gene Transfer Protocol #0107-488: A Phase I, Open-Label Clinical Trial of the Safety and Tolerability of Single Escalating Doses of Autologous CD4 T Cells Transduced With VRX496 in HIV-Positive Subjects

Principal Investigators:	Rob Roy MacGregor, M.D., University of Pennsylvania Medical Center Carl H. June, M.D., University of Pennsylvania Health System
Sponsor:	VIRxSYS Corporation, represented by Boro Dropolic, Ph.D.
RAC Reviewers:	Dr. Aguilar-Cordova, Ms. King, and Dr. Markert
Ad Hoc Reviewers:	Jiing-Kuan Yee, Ph.D., and John A. Zaia, M.D., Division of Virology, City of Hope National Medical Center and Beckman Research Institute John M. Coffin, Ph.D., Tufts University School of Medicine, NCI

A. Protocol Summary

The proposed study is a phase I trial to evaluate a HIV-based lentiviral vector, VRX496, expressing an antisense sequence targeted to HIV *env*. The primary objective of the study is to determine the safety and tolerability of treatment with autologous CD4+ T cells transduced *ex vivo* with VRX496. The vector is derived from HIV sequence but does not code for any viral proteins. VRX496 directly interferes with wild-type HIV (wt-HIV) expression via anti-*env* antisense expression in vector transduced CD4 cells that become infected with wt-HIV. Expression of the anti-HIV antisense *env* from an HIV vector transcript would inhibit wt-HIV RNA to decrease productive HIV replication in the CD4 T cells. The clinical goal for the approach is to decrease viral loads and promote CD4 T cell survival *in vivo.*

Data from *in vitro* studies suggest that HIV vectors such as VRX496 could potentially reduce viral loads in HIV positive individuals, thus delaying the onset of AIDS by promoting CD4 T cell survival and providing the immune system with a better chance to control the infection. Additionally, preliminary results from experiments in severe combined immunodeficiency disease (SCID) mice indicate that the human cells transduced with VRX496 and implanted into the SCID mice do not cause adverse effects.

HIV positive patients will undergo leukopheresis with subsequent CD4 T cell isolation. Patient CD4 T cells will be transduced *ex vivo* with the vector, expanded for 8-11 days, and then the modified cells will be reintroduced into the patient. Each participant will receive a single intravenous injection infused over 30 minutes; participants will be examined 24, 48, and 72 hours post-injection and weekly for 4 weeks. Patients will receive one of four different ascending doses (1×10^9, 3×10^9, 1×10^{10}, and 3×10^{10} cells). Doses will be administered to four independent, sequential subject cohorts of 3 patients. Groups will be administered escalating doses at 6-week intervals after safety has been demonstrated in the previous group. Follow-up examinations will be conducted 1, 3, and 6 months post-injection. Long term follow-up including replication competent lentivirus (RCL) testing will be performed.

B. Reviews by RAC Members and *Ad Hoc* Reviewers

Seven RAC members voted to review this protocol publicly, primarily because of the novel vector. Ms. King, and Dr. Markert submitted written reviews, as did *ad hoc* reviewers Drs. Yee and Zaia, to which the investigators responded in writing and during this meeting. Drs. Aguilar-Cordova and Coffin presented oral reviews at the meeting.

Dr. Aguilar-Cordova focused on the vector product. While commending the investigators for developing a new vector platform, he noted that the SCID mouse model may not be an appropriate model since HIV replication cannot occur within mouse cells. He expressed doubt about the accuracy of their statement that because the vector contains only HIV sequence (with a short tag sequence), recombination can not lead to the generation of a more pathogenic virus. Because of the sequence diversity among HIV isolates, recombination with the vector could generate a virus with a different phenotype than the wt HIV virus that infected the research participant. It would be important to analyze any mobilized genomes. While the proposed RCL assays will detect a full Vesicular Stomatis Virus G (VSVG) pseudotyped lentiviral recombinant, partial recombinants that may occur at the RNA level in the virion would not be detected. Dr. Aguilar-Cordova also questioned the safety of a two plasmid vector

system compared to the multiple plasmid systems. Most of the challenge experiments conducted by the researchers used low concentrations of HIV. He asked how the low concentration of HIV used related to the levels expected *in vivo* in the serum of the research participant or the reservoirs found in lymph nodes and other relevant sites.

Dr. Markert focused on the rationale, design, and safety of the protocol. She noted some problems with the animal data—for example, a variety of liver enzymes that were fluctuating—and suggested that the entire animal data be reviewed when they are available. She also suggested that the sponsor should further characterize the nature of the animals' focal lesions noted in the toxicology studies. Her other suggestions included an evaluation of T-cell diversity prior to entry into the protocol since expanding T cells with a limited diversity would not be helpful to the research participant. Therefore, tests should be done prior to enrollment to screen for participants with a reasonable repertoire of T cells (immunoscopic evaluation of research participants prior to entry with rechecks throughout the study, and participants should also be checked every 6 months for a proliferative response to tetanus). The definition of "failing HAART" required clarification. Dr. Markert also suggested that the DSMB should meet if there is a change in the CD4 cell count and/or the plasma RNA level, and not merely at specified time points.

Ms. King focused on safety, the risk-benefit balance, and the informed consent document and process. She requested a clarification of the study population, the treatment alternatives available to these research participants, more information about the anticipated risk-benefit balance for each dosing cohort, and a description of the data and safety monitoring plan. She questioned why the researchers believe it is ethically appropriate to move from preclinical to clinical studies at this time and with this design. Ms. King suggested an independent assessment be done to ensure that research participants do not have reasonable standard alternatives to participating in this trial. The definition of "failing HAART" needs more discussion in the informed consent form. The Alternatives section in the informed consent document needs additional work, as do the Purpose and Benefits sections.

Dr. Yee expressed concern about safety of a vector production system that uses only two plasmids in 293 cells, and he asked about the rationale for not using a four plasmid system in 293T cells. He stressed that sensitive assays for RCL detection should be developed to detect gag/pol recombinants. Another potential recombination problem could occur if vector system VSVG sequence becomes integrated in cells. If these cells later become infected, HIV could be pseudotyped with VSVG and then infect a broad range of cells. Dr. Yee also noted that wt-HIV does not replicate well in mouse cells, thus limiting the utility of mouse models for biodistribution studies.

Dr. Zaia focused on the study design. He suggested that instead of a dose escalation study, vector safety data might best be captured by using a single dose with an increased observation period. Dr. Zaia stated that it is highly unlikely that a change in viral load and stability of CD4 cells will be detected using an infusion of the proposed number of cells. He suggested testing for survival of the transduced cells compared to cells transduced with a null vector. He asked about the nature of the outgrowth of virus observed after two weeks of culture *in vitro*. He expressed concern that pulmonary toxicity might occur and that this possibility may not have been addressed adequately. Dr. Zaia also expressed concern about the chance of affecting the virus outcome by modifying the peripheral blood, which represents approximately 1 percent of the total body CD4s.

Dr. Coffin also expressed concern about the breakthrough virus and suggested the study be extended to ensure detection of any breakthrough mutants. The mechanism of *env* antisense inhibition is unknown; therefore, the number and types of wt *env* mutations required to create a resistant virus is also unknown. Follow-up studies should be done to determine whether resistant virus emerges in the research participants. Because wt HIV is likely to be slightly different in each research participant, possible recombination with the vector (e.g. repair of a defective long terminal repeat in the wt HIV) could change the wt HIV in the research participant and affect disease outcome. He pointed out that if research participants are able to continue on preexisting HAART during the time their cells are

harvested, it will be difficult to wash out all the drugs from the cells to make certain that those cells are effectively infected with the experimental vector.

C. RAC Discussion

The following additional comments were raised:

- Dr. Noguchi asked several technical questions regarding VSVG detection and pointed out that in any production system there is an inherent limit of sensitivity to the substance being assayed. So one can state only that it is not detectable by the techniques being used at the time, not that the substance is not present.

D. Investigator Response

Dr. June clarified Dr. Zaia's concern about the potential conflict of interest when the investigator who has a proprietary interest in the study method is also responsible for the quality assurance and release testing of the cells. The University of Pennsylvania has established a quality-assurance program that is external to the cell production and uses good laboratory practices-based, quality-control release criteria. Many release criteria will be in place for this protocol with oversight by an external quality-assurance provider.

Dr. MacGregor explained the criteria for including research participants in the trial: individuals who have been treated with several different regimens that have not been effective in controling virus production such that the CD4 cell counts are close to or at (but not below) 200. His current clinical group contains 15 or 20 such patients. "Failure of treatment" would be defined as ongoing viral replication despite antiviral treatment.

Dr. June responded to Dr. Zaia's concern about sampling peripheral blood by explaining that 30 seconds is the average resonance time of a T cell in the peripheral blood before that cell returns to the lymph node. A peripheral blood sample at one point in time will contain a mixture of all the various types of T cells, and this method represents the only practical way to accomplish the goals of this study other than infusing stem cells.

Regarding partial VSVG recombinants, Dr. Dropulic explained that the investigators will use release-testing criteria to demonstrate no detection of VSVG DNA or RNA sequences in the final product. Investigators will use very sensitive assays, including TaqMan (real-time) polymerase chain reaction.

With regard to the adequacy of the SCID mouse studies, Dr. Dropulic restated that although they only detect a single event and have some limitations in sensitivity, they showed no significant RCL-type mobilization and represent the best possible animal model.

Dr. Dropulic indicated that he and his colleagues were unaware of evidence showing that four-plasmid systems are superior to two-plasmid systems. The 293 cells were being used instead of 293T cells because that cell line could be traced and the serum used documented as coming from bovine spongiform encephaly-free countries. As a safety modification, the two-plasmid system proposed for this trial will include pause sites to prevent read-through.

E. Public Comment

Dr. Beth Hutchins, Canji, Inc. (speaking as a member of the public), expressed her concern that the product and its risks are not being represented accurately. The product is identified as a gene transfer product, not as a viral vector or as a new vector class, and the risks listed in the protocol are incomplete. Clarifying the nature of the product in the informed consent document, not just within the oral consenting process, would be the most straightforward approach.

F. RAC Recommendations

Dr. Mickelson summarized the following RAC recommendations, suggestions, and comments.

With regard to pre-clinical studies:

- Stricter testing for VSVG prior to lot release should be implemented.
- Further details about the focal lesions seen in several animals should be provided. Depending on the nature of these lesions, revisions to the clinical protocol and informed consent may be necessary.
- Samples of all clinical lots of the vector should be archived.
- Concerns about the use of 293 versus 293T cells and the use of a 2-plasmid versus a 4-plasmid production system should be considered.

With regard to the clinical protocol:

- Entry criteria about what constitutes a "HAART failure" should be clarified. The investigator should consider consulting with another HIV clinician to confirm that no other acceptable antiretroviral regimen is available as an alternative to study participation. Further details should be added as to which antiretroviral medications can be taken during the course of the study.
- The proposed addition of T-cell repertoire testing by immunoscope technology should occur before administration of the vector product and at one point 4 to 12 weeks post-therapy. Responses to tetanus vaccine should be monitored, as outlined in the modified protocol.
- The proposed 4-week follow-up period prior to advancing to the next subject was discussed extensively, with particular focus on whether 4 weeks would be long enough to confirm the presence of a new strain of lentivirus in the participant. The potential that new viral strains will develop and take longer than the proposed timeframe to appear was also discussed. These concerns should be discussed in both the protocol and the informed consent document and addressed in the sponsor's response to these recommendations.

With regard to the informed consent document:

- It should be clearly stated that this is the first clinical use of a new vector class.
- The concerns surrounding this vector class should be explained.
- "HAART failure" should be defined more clearly. The antiretrovirals that may be taken during the study, as well as those that may not, should be listed.
- The second paragraph of the "Purpose" section of the revised informed consent document should be made into a new section entitled "Possible Benefit." It should replace the current "Benefits" section.

With regard to broader safety issues:

- It is recommended that the definitions of which adverse events would lead to DSMB review should be better defined. For example, in order to establish what would constitute a significant decrease in CD4+ cell counts or increase in viral load, the participants' normal variability in these counts should be established over a prolonged period of time (such as 6 months or even one year prior to study enrollment).
- Specific assays with which to monitor survival of transduced cells should be developed.
- To reduce the possibility of generating a potentially more virulent HIV virus than would already be present in the test subjects, individuals with only CCR5-utilizing strains of HIV should be excluded from the study.
- Specific tests to assess the genetic interaction between the vector and the resident HIV strain should be developed. Such tests could include assays for degenerate gag/pol

sequences; changes to mobilized vector and recombinant genomes; and the HIV viral genotype pre- and post-gene transfer.

G. Committee Motion 4

As moved by Dr. Gordon and seconded by Dr. Aguilar-Cordova, these recommendations were approved by a vote of 9 in favor, 0 opposed, and 0 abstentions.

VII. Data Management Report/Dr. Greenblatt

Dr. Greenblatt reported that a total of 484 gene transfer research (GTR) protocols have been submitted to the Office of Biotechnology Activities (OBA) since June 1988; 20 new protocols were submitted to the OBA in the past 3-month reporting period: 4 were determined by the RAC to warrant public review. 443 GTR protocols are aimed at the development of a therapeutic approach. Of these,

- 303 are for cancer.
- 52 are for monogenic diseases (CF [21] and hemophilia [5] were the most numerous).
- 37 are for infectious diseases (36 for HIV and 1 for Epstein-Barr virus infection).
- 51 are for other diseases (coronary artery disease [19] and peripheral artery disease [16] are the most numerous).

A. Amendments and Updates

The amendments and updates submitted to OBA in the past reporting period involved changes in PIs or trial sites, annual updates, notification of trial closure, annual reports as submitted to FDA, annual IRB approvals, clarification of long-term followup, amendments to change the definition of the maximum tolerated dose, changes in route of administration, and changes of the study agent name. Three reports were highlighted.

OBA received one notice of suspension of a study. Protocol 9902-285, a Phase I trial of intratumoral antisense liposomes for advanced oral squamous cell carcinoma, was suspended by the investigator on July 23, 2001 after it was discovered that research participants were given the wrong plasmid in combination with the liposomes due to an error in the sequence of the gene transfer product. The clinical site has initiated two audits—one audit to review the medical records to validate that no toxicities related to the transfer were observed and a second audit to review the procedures in the laboratory that produced the plasmid. The DSMB reviewed the issue. The study had accrued 13 research participants, 10 of whom had completed dosing.

Another report related to Protocol 0007-407, a Phase I study of gene transfer administration by intramyocardial injection during coronary artery bypass grafting (CABG) surgery in patients with areas of viable and underperfused myocardium not amenable to bypass grafting or percutaneous intervention. The protocol required research participants to have open heart bypass surgery followed by vector administration. In one case, during surgery, it was determined that a research participant who was not a candidate for CABG surgery was still administered the vector. The sponsor is reviewing with FDA whether inclusion criteria should be changed. Meanwhile, the sponsor has reminded all investigators on their study that the protocol does not permit administration of the study agent unless the participant is undergoing CABG surgery.

A third report related to Protocol 9902-292, which involves the use of a recombinant fowl pox vector to immunize research participants with metastatic melanoma. A notification was received that a participant

who did not have the correct human leukocyte antigen (HLA) type was enrolled in the study. The eligibility criteria called for HLA 0201; the patient was HLA 0205. Error was attributed to the fact that the investigator has many other protocols that are HLA A2 specific, and that this was the investigator's first protocol that required a more specific HLA type. The investigator discussed the error, and additional steps were taken to avoid the error in the future.

In all these cases, the investigator indicated that none of the participants was harmed by the mistakes.

B. Adverse Events

Analysis of AE reporting for the period May 1 to August 1, 2001 indicated that, of the 199 serious or unexpected reports submitted to OBA, 140 were initial reports and 59 were follow-up reports. Of the 20 reports that were classified as serious, possibly associated, and unexpected, one was determined to warrant discussion.

That AE occurred on Protocol 0001-372, a Phase I, single-dose escalation study of minimal (gutted) adenovirus expressing Factor VIII for research participants with severe hemophilia A. Approximately five hours after intravenous infusion of the vector, the research participant developed fever, chills, achiness, back pain, and headache. The fever peaked at 102.6 degrees Fahrenheit approximately eight hours after vector infusion infusion and resolved by about twelve hours. On Day 1 post-infusion of the vector, the research participant experienced a spontaneous hemarthrosis of the knee which was treated with recombinant Factor VIII in the usual manner with resolution of the bleeding event. The research participant has a history of multiple spontaneous bleeds. The research participant also experienced elevation of liver enzyme values that peaked on Day 7 post-infusion and transient declines in Factor VII levels and platelet counts. All laboratory values returned to baseline by Day 19 and were not considered by the investigator or sponsor to be clinically significant. Dr. Patterson additionally noted that twenty-four hours after vector administration there was an elevation in fibrin split products.

Dr. Gordon cited a paper from Inder Verma's lab showing that non-infectious, psoralen UV cross-linked adenovirus elicited toxic reactions in animals and noted that this could be instructive if a causative relationship were truly established. It could possibly be secondary to an immune response in an already immune subject receiving a high load of an immunogen or possibly cell surface interactions with hepatocytes and the vector. With respect to the participant's knee hemarthrosis, Dr. Gordon noted that in addition to an underlying Factor VIII deficiency, a consumptive coagulopathy, as suggested by the elevated fibrin split products and lowered Factor VII levels, may have facilitated the spontaneous bleed.

Given the nature of the response in the first research participant to receive this vector, a subgroup of the RAC, composed of Drs. Aguilar-Cordova, Ando, Breakefield, Greenblatt, and Markert, had reviewed the event in greater depth. This group discussed the laboratory findings, the transient nature of the reaction, and the viral titers. The RAC subgroup requested that the sponsor provide additional information on the viral stocks used in the nonhuman primate studies. Also, some discussion focused on whether future research participants should be pre-treated with steroids to decrease any inflammatory response.

Dr. Patterson explained that OBA has been in dialog with the Sponsor (GenStar) about additional clinical and preclinical data being gathered. OBA requested that GenStar present the lessons learned and a comprehensive assessment of this event at a future RAC meeting. GenStar has agreed to make such a presentation.

Dr. Simek explained that FDA is in the process of developing an adenoviral reference standard based on a wild-type virus. This material has been manufactured and is being sent for testing. The material should be ready for use in the first week of March 2002. Dr. Noguchi and Dr. Anne M. Pilaro, FDA, explained that FDA is in consultation with GenStar regarding additional steps to be taken.

Ms. Levi-Pearl stated that she was encouraged by this kind of feedback to the RAC, and she commended OBA for moving quickly, obtaining more information, and forming a subgroup of the RAC to evaluate information in more detail. Additionally, she expressed concern that the informed consent document for this study should be revised to reflect this new clinical information.

VIII. Communication of Issues Raised by Individual RAC Members Following the Preliminary RAC Review of Human Gene Transfer Protocols: Followup Discussion/Ms. King

This issue was introduced at the June 2001 RAC meeting. RAC members agreed to the proposal in principle and requested that draft language be developed for RAC review and approval at a subsequent meeting. Ms. King prepared draft language, which was circulated to the RAC for review and modification prior to this meeting. The language reads as follows:

> "As you know, during the preliminary protocol review process, individual RAC members may request additional information or clarification about your protocol and sometimes make specific comments or suggestions about the protocol design, informed consent document, or other matters. Any such individual RAC member questions or comments are then conveyed to you. All such correspondence is part of the public record of this protocol and is available to you and your IRB and IBC, upon request, for your assistance during local review of your protocol. It is important to emphasize that the comments of individual RAC members about your protocol, while representing the considered perspective of one or more members with knowledge and experience in this area, do not constitute a consensus of the RAC, nor are they in any way binding on you or your institution. Copies of the correspondence may be obtained by requesting the RAC comments for your protocol through the general OBA email address (oba@oba.nih.gov<mailto:>) or by calling 301-496-9838. We hope that this service will be of value during local review of your protocol."

The language would be included in the letters that OBA sends to PIs, IRBs, IBCs, sponsors, and FDA regarding protocols that are exempted from in depth RAC review and public discussion.

Dr. Markert suggested removing the last sentence. Dr. Gordon suggested trying this approach for 6 months. Dr. Mickelson noted that all of the information that is presented during the preliminary review of protocols is part of the public record.

A. Committee Motion 5

As moved by Dr. Gordon and seconded by Dr. Mickelson, this language and process including an evaluation after six months, was approved by the RAC by a vote of 10 in favor, 0 opposed, and 0 abstentions.

IX. Proposed Response to Reported Appearance of Neoplasms After Vascular Growth Factor Gene Transfer/Dr. Gordon

Dr. Gordon explained that the RAC has received reports in the recent past of malignancies in research participants who received vascular growth factor (VGF) gene transfer. While the RAC has reacted to individual events, and the issue was discussed as part of the cardiovascular gene transfer safety symposium, the committee has not developed a formal response to the issue. The public interest warrants a formal response.

Dr. Gordon proposed that a reasonable approach would be to screen patients for incipient malignancies prior to gene transfer. "Reasonable screening" should be relatively inexpensive and include the recommended screening for each research participant's age or risk group. However, there is no consensus regarding screening recommendations. Dr. Gordon summarized the screening recommendations of NCI,

the American Cancer Society, and the U.S. Preventive Services Task Force. Another issue is whether recipients of VGF gene transfer should be considered high risk for developing tumors. Research participants who are considered to be in a high-risk category may require different types of tests, different frequencies of screening, and screening should be provided at different ages. Dr. Gordon stated that, in his opinion, research participants who receive VGF gene transfer should be considered at relatively high risk.

Dr. Gordon offered a proposal for RAC consideration: VGF gene transfer recipients should be screened for malignancies prior to enrolllment. He read into the record proposed language that would be included in the OBA "approval" letter sent to the PIs and sponsors of VGF gene transfer protocols:

> "Several cases of tumors arising after vascular growth factor gene transfer have been reported to the RAC. A causal relationship between gene transfer and tumor development has not been scientifically established. However, our present understanding of the action of these growth factors and the relationship between their tumor vascularization and progression are consistent with a model where tumor production is stimulated by compounds that increase tissue vascularization. For these reasons, we request that patients being admitted to clinical trials involving vascular growth factor gene transfer undergo cancer screening prior to the trial. Appended to this letter is a list (developed in consultation with NCI) of recommended screening procedures."

Promoting such screening may help enhance safety and provide an opportunity to gather useful information. Dr. Gordon cautioned that care should be taken so these screenings do not become an inducement to participate in a study while noting the benefit of such screening procedures for the participants.

A. RAC Discussion

Dr. Ando asked whether genetic screening also should be considered. Dr. Greenblatt suggested that any tumors that are found in research participants receiving VGF gene transfer should be tested for vector sequences. Ms. King suggested that screening be standardized, that a safety conference be organized to examine instances in which tumors developed and to assess how to respond, and that more studies in animal models be encouraged to determine the effect, if any, of VGF on preexisting or naturally occurring tumors. Dr. Friedmann was not concerned about the lack of screening consensus because the group under consideration represents a narrow population that is easier to define. He asked whether anyone is keeping a tally (anecdotally noted) of instances of tumor development in research participants undergoing growth factor gene transfer. Dr. Breakefield suggested that the RAC recommend to FDA that a standard for screening be set. Dr. Greenblatt proposed that the wording simply state "cancer screening" without specifying particular tests.

Dr. Gordon proposed that the RAC appoint one or more RAC members to look into the possibility of creating basic screening guidelines for research participants in VGF gene transfer studies. The role of this *ad hoc* group would be to confer with experts in this field and report back to the RAC on whether a reasonable core list of screening tests exists or can be developed.

Dr. Noguchi provided the FDA perspective on this issue, which has not been to develop a standard list of required screenings before starting a protocol since each protocol is slightly different. A person who has cancer or a propensity to cancer should not be entered in a growth factor trial.

Dr. Aguilar-Cordova suggested a possible recommendation to the NIH Director about the advisability of studying this issue prospectively, perhaps through issuance of a Request for Applications. He also suggested that a toxicologist review the proposed language for the recommended screenings.

B. Public Comment

Joann C. Delenick, Arlington, VA, queried whether these screening panels would be covered by Medicare or Medicaid. She suggested adding wording about eventual autopsy.

C. Conclusion of Discussion

Drs. Gordon and Greenblatt volunteered to investigate this issue further to provide the RAC with more clear direction. Included in their report to the RAC will be information about how many protocols already contain this language regarding screening.

X. Day One Adjournment/Dr. Mickelson

Dr. Mickelson thanked the participants and adjourned the first day of the September 2001 RAC meeting at 6:20 p.m. on September 6, 2001.

XI. Day Two Opening Remarks/Dr. Mickelson

Dr. Mickelson opened the second day of the September 2001 RAC meeting at 8:30 a.m. on September 7, 2001.

XII. Discussion of Human Gene Transfer Protocol #0107-487: An Open-Label, Phase I, Single-Administration, Dose-Escalation Study of AD_{GV}PEDF.11D (ADPEDF) in Neovascular Age-Related Macular Degeneration

Principal Investigator:	Peter A. Campochiaro, M.D., Johns Hopkins University School of Medicine
Sponsor:	GenVec Inc., represented by Henrik S. Rasmussen, M.D., Ph.D.
RAC Reviewers:	Drs. Breakefield, Chow, and Mickelson
Ad Hoc Reviewers:	Martin Friedlander, M.D., Ph.D., Scripps Research Institute Joan Schwartz, Ph.D., National Institute of Neurological Disorders and Stroke Marco Zarbin, M.D., Ph.D., University of Medicine and Dentistry of New Jersey

A. Protocol Summary

Age-related macular degeneration (AMD) is one of the two most common causes of vision loss among adults in the United States and other developed countries. In the United States at least 1.7 million people have impaired vision resulting from AMD. Every year more than 165,000 people contract AMD, and 16,000 go blind from it, predominantly from a rapidly progressing form of the disease called “wet” AMD. In wet AMD severe vision loss is caused by abnormal blood vessel growth in or around the retina and subsequent vessel leakage. In functional terms, people with wet AMD are unable to read, recognize faces or drive, and the disease often leads to legal blindness. The onset of severe visual changes in wet AMD can occur suddenly. More than 400,000 Americans are currently affected by this form of the disease, and the incidence is rising rapidly as the U.S. population ages. The serious consequences of this disease—along with limited treatment options that offer limited effectiveness—make AMD a good candidate for a gene transfer treatment approach.

The replication incompetent adenoviral vector, AD_{GV}PEDF.11D, is designed to transport the gene for human pigment epithelium-derived factor (PEDF) into relevant cells in the eye. PEDF is one of the most potent known inhibitors of blood vessel growth found in humans. As the eye creates a natural barrier between itself and surrounding tissues, it is unlikely that AD_{GV}PEDF.11D would affect tissues other than the eye.

Administration of AD_{GV}PEDF.11D directly into the eye provides a convenient means of delivering PEDF to the cells of the eye and is likely to result in a longer duration of effect compared with administration of PEDF as a protein alone.

In three mouse models of a disease similar to AMD, significant inhibition (up to 85 percent) of new blood vessel growth was demonstrated with doses of AD_{GV}PEDF.11D ranging from $1x10^8$ to $1x10^9$ particle units. In safety studies performed in monkeys, these doses were well tolerated with no toxicity observed at a dose of $1x10^8$ pu and only minimal and reversible inflammatory responses at $1x10^9$ pu. Higher doses showed more severe inflammatory responses.

The proposed clinical investigation is an initial study of the safety of gradually increasing doses of AD_{GV}PEDF.11D injected into the eye. In addition, researchers will look for any potential effects this compound has on vision. Research participants will be age 50 or older and will have severe wet AMD in at least one eye.

B. Reviews by RAC Members and *Ad Hoc* Reviewers

Five RAC members voted to review this protocol publicly. Drs. Breakefield and Chow submitted written reviews, as did *ad hoc* reviewers Drs. Friedlander, Schwartz, and Zarbin, to which the investigators responded in writing and during this meeting.

Dr. Breakefield expressed concern that the gene transfer experiment could accelerate eye damage in participants through vector-related toxicity or effects of PEDF. She also questioned the choice of vector since Ad vector transgene expression is transient. Dr. Breakefield requested that the investigators provide data on the levels and duration of PEDF expression in the eye after injection. She asked about capsid toxicity, the likelihood of some level of inflammatory response, leakiness of the eye-blood barrier, whether the 3-week followup is long enough, and which types of monitoring should be conducted. Dr. Breakefield requested that the RAC be shown the monkey data when they are available.

In her absence, Dr. Chow's review was presented by Dr. Mickelson. Dr. Chow's review centered on a concern that there may be more risk in human studies compared with animal studies because of the presence of preexisting antibodies. If steroids are used to deal with an inflammatory response, researchers should consider the possible effect of steroids on neovascularization. Long-term expression of high levels of PEDF in the eyes of AMD patients could be harmful, even if an inflammatory reaction is not at issue. Dr. Chow suggested that investigators consider using a less immunogenic vector, perhaps an AAV vector.

Dr. Schwartz focused on the possibility of an inflammatory response and measurements of PEDF in the eye. She wondered why investigators were not detecting PEDF in the normal eye and requested a description of the enzyme-linked immunosorbent assay (ELISA) being developed. Dr. Schwartz asked the investigators to provide information about how long the immune response was monitored after administration of the PEDF-containing vector in each of their studies and which specific criteria were used to monitor the immune response in the eye and systemically. She wondered whether repeat injections would lead to increased immune problems.

Dr. Friedlander noted that potential research participants have much to lose if this trial has a negative effect on their vision. They could lose their ambulatory vision, which allows them to care for themselves. Dr. Friedlander asked whether the investigators had considered using other vectors such as AAV or gutless AdV. The proposed vector system may not be the most appropriate for the disease model since expression will be transient while the proliferative retinopathy will be lifelong. In regard to PEDF expression, he asked the following questions: What is the exact mechanism of action of this molecule in the eye? Are there data that address whether the level of PEDF in eyes affected by peripheral retinopathies is different than in unaffected eyes? Does a PEDF knockout mouse model exist and if so, what has it revealed regarding the molecular mechanism? What is the potential effect of PEDF on existing vessels, such as causing collateralization? Can PEDF levels be adequately measured in the eye, and can this methodology

be used to determine whether PEDF escapes into the adjoining brain or systemic vasculature? What are the pharmacodynamics of PEDF in the eye?

Dr. Zarbin participated in this RAC meeting via conference call. Dr. Zarbin focused on the fact that inflammation and retinal damage have been observed at doses of $1x10^{10}$ pu in cynomologous monkeys. The toxicity studies have been conducted in primates that do not have choroidal neovascularization (CNV) while the efficacy studies have been conducted in rodents that do have CNVs. The rationale of the choice of 4 weeks as the minimal time interval in which the study eye was not to have received any other treatment for AMD was not clear. In one part of the protocol, the investigators propose to perform intravitreal injections under topical anesthesia, which Dr. Zarbin suggested should be replaced with retrobulbar anesthesia. He asked how long the first, lowest dosed research participant will be observed before the trial proceeds to the next participant. In regard to potential development of glaucoma, Dr. Zarbin questioned whether the results of the intravitreal-injected animal experiments would be predictive of any effects in humans because of the differences in the ratio of the size of the lens to the volume of the vitreous chamber.

C. RAC Discussion

Dr. Markert asked the investigators whether any of the monkeys had been immunized to adenovirus to more closely mimic the human situation (i.e., has a non-naive animal model been used). She also noted that improvements need to be made to the informed consent document, especially the long paragraph about risk. Stronger statements about the potential for risk should be included.

Dr. Breakefield asked whether intravitreal injections were the best strategy. Dr. Zarbin stated that patient discomfort with this procedure should be the guiding factor. Dr. Campochiaro explained that intravitreal injections under topical anesthesia is the standard method in his laboratory. Dr. Breakefield also suggested increasing the length of the observation period after the procedure given the possible responses to the gene transfer. Dr. Zarbin concurred, suggesting that the window of observation be extended and that one research participant be dosed at a time.

D. Investigator Response

Dr. Campochiaro reiterated that CNV is one of the most significant problems faced by retina specialists. Current treatments, such as laser, are particularly destructive. The trial proposes vector administration in one eye only, thus leaving peripheral vision in the other eye unaffected. Inflammation is the primary concern regarding adverse effects, but Dr. Campochiaro noted that inflammation is a relatively common occurrence in CNV patients, is treatable, and generally does not result in permanent loss of vision.

Regarding length of PEDF expression, Dr. Campochiaro described an ELISA currently being used, but data are not yet available as to how long and at exactly what level expression occurs. It is known that there is high-level expression compared with baseline and that PEDF expression is at least tenfold higher after subretinal injection compared with intravitreal injection. Initial data suggest that expression decreases significantly by 1 month after transduction.

Responding to suggestions about the use of AAV vectors instead of the proposed adenoviral vector, Dr. Campochiaro explained that AAV vectors have produced inhibition in the models tested to date. However, the level of inhibition is lower, likely because of the lower level of expression. Dr. Rasmussen responded to concerns about the vector system. He explained that because the long-term toxicity of PEDF is not known, it may be preferable to use a transient vector system. An AAV vector might express PEDF for months or years.

Spontaneous regression does not occur in the CNV model or the VEGF model. Dr. Campochiaro explained that the investigators had looked out to 4 months post treatment in mice and monkeys and had seen no regression. In animal models, no transduction has been detected outside the eye.

Dr. Rasmussen clarified that all research participants will be followed for as long as 1 year to detect AEs. The choice of a 3-week interval between dosing cohorts was based on the monkey data: when toxicity was detected, it had occurred within the first week and peaked after approximately 2 weeks, with no further adverse reactions observed after 3 weeks. He offered to increase the interval between cohorts to 4, 5, or 6 weeks if the RAC felt strongly about this issue.

Through ophthalmologic examinations, Dr. Rasmussen and his colleagues are convinced that they will be able to detect even slight signs of inflammation. Detection of any inflammation would preclude proceeding to a higher dose. From the animal studies, the investigators have determined that steroid treatment may be helpful in ameliorating inflammatory responses that might occur.

E. Public Comment

Dr. Lori Ellis, The Foundation Fighting Blindness, noted that one in four individuals older than 65 will have AMD in some form and that half of individuals older than 85 suffer from AMD, and that the foundation strongly advocates the development of safe and effective treatments to restore vision, improve the quality of life, and reduce the societal costs brought about by inherited retinal degenerative diseases.

Harriet L. Finkelstein, Vice Chair of National Board of Trustees for The Foundation Fighting Blindness, read a statement that described the impact that inherited retinal degenerative disease has had on her family. Her father was diagnosed with AMD 15 years ago. A proud and independent man, he was robbed by AMD of his ability to drive and participate in the activities he enjoyed, which changed his quality of life dramatically. Ms. Finkelstein stated her belief that her father's inability to participate in life as he viewed it hastened his death. Because AMD is a genetic disease, she is concerned that AMD will one day affect her and her siblings. Ms. Finkelstein expressed full support of efforts to develop safe and effective therapies, including gene transfer, to arrest AMD.

F. RAC Recommendations

Dr. Mickelson summarized the following RAC recommendations, and comments. Investigators should address the following observations and suggestions:

With regard to pre-clinical studies:

- A third animal study has been started and the results are anticipated prior to the start of the human trial. Since this non-human primate study utilizes a disrupted Bruch's membrane, it has the potential to serve as a good model for the leaky membranes found in age-related macular degeneration. Additional information should be gathered regarding the potential release of the gene transfer product into the vasculature and the central nervous system. The third animal study should begin to address these issues.
- Samples of all clinical lots of the vector should be archived.
- Further development of Dr. Campochiaro's ELISA assay specific for PEDF is important and will enable assessment of gene product levels in experimental animals and human subjects.
- The assay could potentially enable the investigators to answer important questions such as the following:

    1. After intravitreal application of the gene transfer product, what is the level and distribution of the product in the eye and its vasculature? What is the rate of elimination?
    2. How do intravitreal PEDF levels differ between people with and without age-related macular degeneration?
    3. What is the proposed mechanism of action of PEDF in regard to its ability to reverse macular neovascularization? Why does it target only new vessels?

With regard to the clinical protocol:

- The use of retrobulbar anesthesia instead of topical should be considered.
- Baseline anti-adenoviral antibody levels should be obtained.
- Risks and benefits of study participation should be clarified in the informed consent document. Worse case scenarios, such as blindness in the treated eye, should be included in the document and described in lay language.

G. Committee Motion 6

As moved by Dr. Gordon and seconded by Dr. Markert, these recommendations were approved by the RAC by a vote of 9 in favor, 0 opposed, and 0 abstentions.

XIII. Update on the RAC Scope of the *Guidelines* Working Group/Drs. Ando and Patterson

Dr. Juengst chairs the *Guidelines* working group consisting of six RAC members and one *ex officio* member. Dr. Ando explained the charge to the working group, which is to assess the present scope of the *NIH Guidelines*, review the need for public discussion of the newer technologies, and if necessary, suggest changes. Dr. Patterson explained that to provide the committee with information, OBA will survey NIH Institutes and Centers (ICs) about various technologies "in the pipeline" that are intended to modify the human genome. Other agencies such as FDA, Centers for Disease Control (CDC), and Environmental Protection Agency (EPA) would also be consulted. The Working group discussions included emerging technologies ranging from synthetic oligonucleotides to the insertion of a whole nucleus into another cell. The working group considered a conference to discuss the new technologies and the development of guiding principles to include in the *NIH Guidelines* rather than addressing each new technology separately. Dr. Gordon described a possible movement towards a more flexible definition of the *NIH Guidelines* with the RAC's contribution as a public forum to discuss new technologies. He predicted that a more fluid and close interaction between FDA and the RAC will take place in the near future, thus facilitating the RAC bringing these subjects to public discussion.

Dr. Mickelson noted that the next steps would be to generate a list of new and emerging technologies, possibly at a gene transfer policy conference. Involving NIH ICs as well as FDA and Federal agencies would prove beneficial.

XIV. Update on the Vaccine Working Group/Drs. Breakefield and Mickelson

Dr. Breakefield noted that because the *NIH Guidelines* exempt some vaccines from RAC review, the RAC is not made aware of some vaccine trials that involve vectors typically used in gene transfer studies. A working group was formed to discuss the possible effects of modifying the vaccine exemption. Among the effects discussed were delays in implementation of new vaccination strategies for critical diseases, loss of trade secrets or inadvertent breach of confidentiality, an unmanageable increase in the number of protocols to be reviewed by RAC members, and the potential for duplication of review by other Federal government agencies. Possible advantages could be the extensive expertise the RAC has in viral vectors, the recognized public forum provided by RAC review, and the opportunity to interact with other review groups. In addition, the inclusion of AE reports from vaccine trials in the OBA database would provide more complete information about the use of the vector types also used in gene transfer.

Dr. Patterson explained that NIH is keenly interested in furthering the development of vaccines and research to treat a host of human disorders through vaccination. The Vaccine Working Group should recommend a value-added process that will be synergistic rather than duplicative. Many vaccine trials are generating data that could inform future RAC discussions of studies that use the same vectors, and patients, research participants, and scientists could benefit from the results of these public discussions. NIH welcomes input

from FDA, CDC, and members of the public.

Dr. Friedmann suggested that the RAC could play a more active role in requesting information about new technologies or suggesting funding opportunities across NIH ICs. Dr. Patterson noted that two years ago NIH formed a trans-NIH group with representatives from each IC to provide expertise and information to OBA.

XV. Amendments to the *NIH Guidelines*

A. Proposed Action on *E. coli* Risk Group Assessment/Eugene Rosenthal, Ph.D., OBA

In January 2001, OBA received a request from the University of Florida to define the risk group for strain B of *Escherichia coli* (E. coli) bacterium. Currently, only the K-12 strain is designated as a risk group 1 agent in the *NIH Guidelines*. Following discussion at the March and June 2001 RAC meetings, a revised proposal was published in the *Federal Register* (66FR 42555) on August 13, 2001, as follows:

A strain of *E. coli* may be designated as a risk group 1 agent if the following criteria are met:

1. does not possess a complete lipopolysaccharide (i.e., lacks the O antigen and has a "rough" colony morphology), and

2. does not carry any active virulence factor (e.g., toxins) or colonization factors and does not carry any genes encoding these factors.

One public comment was received supporting the proposed change but suggesting that the definition is too complex and specific.

Dr. Rosenthal requested the RAC's input on the version published in the *Federal Register* and approval of the wording. The RAC's input and public comments will be provided to the NIH Director, who will make the final decision.

Dr. James Kaper, University of Maryland, explained that the two proposed characteristics are the same as those used to designate *E. coli* K12 as an exempt strain. He had contacted the editorial board of the American Society for Microbiology seeking input on the proposal and received positive comments including one from Dr. Roy Curtis who derived E. coli strain B.

1. Committee Motion 7

As moved by Dr. Gordon and seconded by Dr. Greenblatt, the language was accepted as published in the *Federal Register*. The vote was 7 in favor, 0 opposed, and 1 abstention.

B. Proposed Action To Allow Modification of the Prescribed Number and Expertise of RAC Members/Dr. Patterson

Dr. Patterson presented a brief overview of a proposal to provide the NIH with the flexibility to augment the composition of the RAC. The proposed action was published in the Federal Register and the public comment period is still open. A public teleconference will be arranged for the RAC to vote on the final action.

Three basic changes are proposed: (1) the size of the RAC will be a minimum of 15 voting members with no maximum number specified in the *NIH Guidelines*; (2) the composition of the RAC will include new areas of expertise as needed; and (3) the *NIH Charter* will be the controlling document of the RAC. Flexibility in the size and expertise of the RAC will enhance its review of recombinant DNA research, given the recent

trend toward an increased number of protocols, an expanded scope of clinical indications, an increased array of vectors used for gene delivery, and the need to provide advice on emerging biosafety and ethical issues. Changing the controlling document will clarify NIH's authority to define the RAC's composition and role and allow the NIH Director to modify RAC membership quickly to react to new scientific developments.

Dr. Noguchi asked about the advisability of an upper limit on the number of RAC members. Dr. Patterson responded that OBA recognizes that committees can become unmanageable once they exceed a certain size; however, greater flexibility would be provided by not choosing an arbitrary maximum number of RAC members. A maximum number will be stated in the charter, but not in the *NIH Guidelines*.

Ms. Levi-Pearl suggested at least one additional patient-advocate position be added to the RAC membership.

C. Final Action To Amend the Safety Information Reporting Requirements/Dr. Patterson

Dr. Patterson reported that this final action is in the ultimate stages of clearance. It has cleared NIH and received FDA concurrence and is now awaiting departmental and OMB clearance. The signed Final Action is expected to be published soon.

XVI. Update on the IBC Policy Conference/Allan Shipp, OBA

Mr. Shipp provided an overview of the upcoming IBC Policy Conference, to be held on Friday and Saturday, December 7 and 8, 2001 at the Bethesda Marriott Hotel in Bethesda, MD. As recombinant DNA research has undergone a significant evolution in the 25 years since the *NIH Guidelines* were drafted, nearly every aspect of these guidelines has been revisited, amended or updated in some way except for the provisions related to IBCs. Furthermore, many observers have cited the changing landscape of clinical research with the increasing prevalence of multisite trials and nonacademic research sites—a trend that has led to new IBC arrangements evolving to accommodate changing research paradigms. OBA now receives registrations for IBCs that are not situated in large academic research centers, as was the traditional IBC paradigm. Thus, the conference objectives will be to:

- Take a fresh look at the expectations, roles, and responsibilities of IBCs;
- Apply these expectations, roles, and responsibilities to traditional and nontraditional IBC arrangements; and
- Consider whether the *NIH Guidelines* have kept pace with the current environment or needs amendment.

The format of the meeting will be open attendance, with IBC chairs, members, and administrators invited to participate. No conference fee will be charged, but preregistration will be required.

Mr. Shipp reviewed the proposed agenda. Policy roundtable discussions will cap the 2-day conference and will include invited experts who represent diverse points of view, including industry representatives, investigators, IBC members and chairs, administrators, and biosafety experts. Questions at this conference will explore key policy matters to aid in the exploration of IBC characteristics, apply those characteristics to case examples that reflect arrangements currently in operation, and discuss the IBC provisions of the *NIH Guidelines*.

The next steps will be to post the agenda and the registration form on the OBA Web site in the coming days, disseminate information about the conference via the OBA news listserv, and publicize the conference widely.

XVII. Donation of Ooplasm as a Treatment for Infertility and Its Implication for the RAC/Dr. Gordon

With in vitro fertilization, the ova of older women fertilize at lower rate possibly due to cytoplasmic defects in older ova. In ooplasmic transplantation, cytoplasm is removed from a donor ovum and introduced into the recipient egg by electrofusion or microinjection. Ooplasmic donation involves transfer of mitochondria, which contain genetic material. A recent paper in *Human Reproduction* described a study of several children born after ooplasmic donation in whom DNA polymorphisms specific to the donor mitochondria were detected. The authors stated, "This report is the first case of human germ-line genetic modification resulting in normal healthy children." Dr. Gordon asked the RAC to consider if ooplasmic transplantation resulted in germ-line genetic modification and, if so, should such protocols require RAC review. The RAC should also consider whether ooplasmic transplantation could have adverse effects related to genetic abnormalities of donor mitochondria that would be transmitted through the germ line.

Dr. Gordon discussed the important features of mitochondrial disorders:

- Mitochondrial disorders that result from mutations in mitochondrial DNA are maternally inherited. Mutations in some mitochondria lead to "heteroplasm," a mixture of normal and abnormal mitochondria.

- In the 37 mitochondrial genes, no parts of the genes are removed prior to translation into protein, which means that mutations in these genes are more likely to affect a structural component of the gene.

- Because no DNA repair activity exists in mitochondria, there is a relatively high mutation rate; mitochondrial DNA accumulates potentially deleterious mutations at a higher rate than nuclear DNA.

- The proportion of abnormal mitochondria varies in different cells and changes as cells divide.

- Disease results when the degree of heteroplasmy (mutational load) exceeds a critical level, which may vary among cell types. Mutational load may change within a cell during the life of the affected individual.

About 100 mitochondria-related diseases have been identified with a wide range of syndromes. About 15 per 100,000 people have mitochondrial disease. Different mutations cause different diseases in most cases. One particularly severe mutation in which 1/3 of the mitochondrial genome is deleted results in three distinct syndromes - Kearns Sayre syndrome, progressive external ophthalmoplegia, and Pearson marrow-pancreas syndrome - which cause diverse abnormalities depending on the tissue affected. The abnormal mitochondria in these syndromes result from a clonal expansion suggesting that the deleted mitochondria have a selective advantage. Therefore, if the donor ooplasm has some mutated mitochondria, these may have a selective advantage in the recipient causing mitochondrial disease which if the recipient is female can be passed on to subsequent generations.

Characteristics of traditional germ-line gene insertion that raise concern include physical interaction of donor and recipient DNA (which could lead to insertional disruption of host genes), altered regulation of host genes, acquisition of new genetic traits that could cause abnormal development of disease, irreversible heritable mutations in Mendelian traits, and the fact that the all offspring cells that inherit the inserted genes are affected. Mitochondrial gene insertion differs from somatic gene insertion in that, when mitochondria are inserted into a cell, physical interaction between the donor mitochondrial genetic material and the host genetic material does not occur, and thus insertional mutagenesis would not occur. Altered regulation of host genes could occur secondarily, but structural arrangements of host DNA are not likely. Irreversible heritability, as in chromosomal integration, will not necessarily occur. All cells would not necessarily be affected in the offspring because of heteroplasmy and nonrandom segregation of abnormal mitochondria.

With regard to whether ooplasmic transplantation protocols should be subject to RAC review, Dr. Gordon

concluded that ooplasmic transplantation protocols should not be reviewed by the RAC as gene transfer protocols, at least at the present because they are more akin *to* organ transplantation than gene transfer.

Dr. Noguchi commented that FDA is beginning *to* bring assisted reproductive technologies under its purview. CDC holds the responsibility for monitoring outcomes of *in vitro* fertilization (IVF) technology. However, outcomes are monitored through each individual center that performs IVF, and only the fertilization rates are reported. FDA will be requiring infectious disease recordkeeping and donor screening and, for more novel techniques, investigational new drug applications. FDA considers ooplasmic donation to be gene transfer because it involves more than the normal fertilization of sperm and egg.

A. Informal RAC Agreement

As recommended by Dr. Gordon, RAC members agreed at this time, that RAC review of ooplasmic transplantation is not warranted.

XVIII. Chair's Closing Remarks/Dr. Mickelson

Dr. Mickelson noted that the next RAC meeting is scheduled for December 5-6, 2001 in conjunction with the IBC Policy Conference. The RAC meeting will be held at the same location (Bethesda Marriott Hotel) as the policy conference.

Dr. Patterson explained that new RAC members will undergo a training session and will attend the December 2001 RAC meeting-not as members of the RAC, but as part of an orientation process.

XIX. Adjournment/Dr. Mickelson

Dr. Mickelson adjourned the meeting at 12:30 p.m. on September 7, 2001

[Note: Actions approved by the RAC are considered recommendations *to* the NIH Director: therefore, actions are not considered final until approved by the NIH Director.]

…/s/…

Amy P. Patterson, M.D.
Executive Secretary

I hereby acknowledge that, to the best of my knowledge, the foregoing Minutes and Attachments are accurate and complete .

Date:

…/s/…

Claudia A. Mickelson, Ph.D.
Chair

Attachment I
Committee Roster

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Attachment II
Attendees

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Emilio Arbe, Ark Therapeutics
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Jessica Boehmer, VIRxSYS Corporation
Alan K. Boyd, Ark Therapeutics
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Attachment III
Abbreviations and Acronyms

AAV	adeno-associated virus
AdV	adenoviral vector
AE	adverse event
AMD	age-related macular degeneration
BMT	bone marrow transplantation
CABG	coronary artery bypass grafting
CDC	Centers for Disease Control and Prevention
CF	cystic fibrosis
CNV	choroidal neovascularization
DNA	deoxyribonucleic acid
DSMB	data and safety monitoring board
E. coli	*Escherichia coli* bacterium
ELISA	enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
FDA	U.S. Food and Drug Administration
GTR	gene transfer research
HAART	highly active antiretroviral therapy
HDCT	high-dose chemotherapy
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
IBC	Institutional Biosafety Committee
ICs	NIH Institutes and Centers
IRB	Institutional Review Board
IVF	*in vitro* fertilization
LTR	long terminal repeat
NCI	National Cancer Institute
NIAID	National Institute of Allergy and Infectious Diease
NIH	National Institutes of Health
NIH Guidelines	*NIH Guidelines for Research Involving Recombinant DNA Molecules*
OBA	Office of Biotechnology Activities (formerly ORDA, Office of Recombinant DNA Activities)
OD	Office of the Director, NIH
PEDF	pigment epithelium-derived factor
PI	principal investigator
PTFE	polytetrafluorethylene
pu	particle units
RAC	Recombinant DNA Advisory Committee
RCL	replication-competent lentavirus
RCR	replication-competent retrovirus
RNA	ribonucleic acid
SCID	severe combined immunodeficiency disease
VEGF	vascular endothelial growth factor
VGF	vascular growth factor
VSV	vesicular stomatitis virus, strain G
wt-HIV	wild-type HIV